OCCURRENCE OF "INDUCED" ANTIGENS IN SALMONELLAE ISOLATED FROM MAN

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It is well known that motile cultures of salmonellae when inoculated into semisolid medium containing appropriate H antisera to prevent migration, may develop "induced" or "artificial" H antigens. Organisms which possess these changed antigens spread throughout the medium since the flagella no longer are influenced by the sera which immobilized the original cultures. Among the H antigens most prone to give rise to such changed forms is antigen b, which is changed to an antigen called z_{33} (Edwards and Moran, Proc. Soc. Exptl. Biol. Med., **61,** 242, 1946). This change is irrespective of the serotype in which the b antigen occurs. Thus, a monophasic form of *Salmonella minnesota* (21: b) placed in semisolid medium with anti-b serum will yield a 21: z_{33} form, whereas *Salmonella paratyphi* B (4,5,12: b-1,2) exposed to b and 1,2 sera is likely to yield 4,5,12: z_{33} — 1,2. Other H antigens yield characteristic "induced" forms when placed in contact with appropriate sera (Edwards and Bruner, J. Bacteriol., **38,** 63, 1939).

The natural occurrence of "induced" antigens has been noted only once, when Edwards and Moran (Proc. Soc. Exptl. Biol. Med., **61,** 242, 1946) recognized the 21: z_{33} form among cultures isolated from sewage by A. A. Hajna. Attention is called here to the appearance of these changed forms in man, including persons affected with gastroenteritis.

Within a period of 2 weeks in May 1950, 9 cultures were received from Miss Janie Morris and Miss Alice Brim of the Georgia State Department of Health, to whom we are indebted for the following information: Four of the cultures were from persons in the same town. Three of the cultures were isolated from 3 adults who denied having any recent gastrointestinal symptoms. The fourth culture was isolated from the stool of a 12-year-old boy who had mild diarrhea.

The fifth person from whom the organisms were isolated was a 4-year-old boy with severe diarrhea who resided in a second town. The sixth isolation was from a person of unknown age and sex who was affected with diarrhea when admitted to a state tuberculosis sanatorium. The seventh and eighth cultures were isolated from the stools of 2 asymptomatic female workers in a school lunch room in a fourth town. The ninth culture was isolated in the course of a routine examination of the stools of a known typhoid carrier.

In agglutinin-adsorption tests the organisms were agglutinated with a 21: z_{33} serum, and the H and O agglutinins were removed. The serum had been derived from a monophasic culture of *S. minnesota* (21: b), which had been passed through b serum and thus changed to 21: z_{33}. The cultures were not flocculated by sera for the normal H antigens of *S. minnesota* (b and e,n,x).

The observations indicate the importance of recognizing the changes that may take place in H antigens of known Salmonella serotypes which otherwise might be designated as independent types. The "induced" form has not been recognized in the area since.

3-HYDROXY-2-NAPHTHOIC ACID AS AN INTERMEDIATE IN BACTERIAL DISSIMILATION OF ANTHRACENE

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The authors have previously reported (Rogoff and Wender, J. Bacteriol., **73,** 264, 1957) the isolation of 1-hydroxy-2-naphthoic acid as an intermediate in the breakdown of phenanthrene

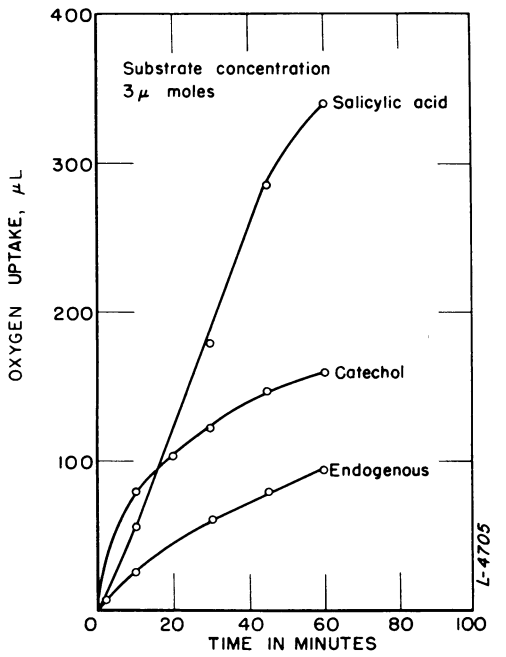

Figure 1. Oxidation of aromatic compounds by anthracene-grown cells.

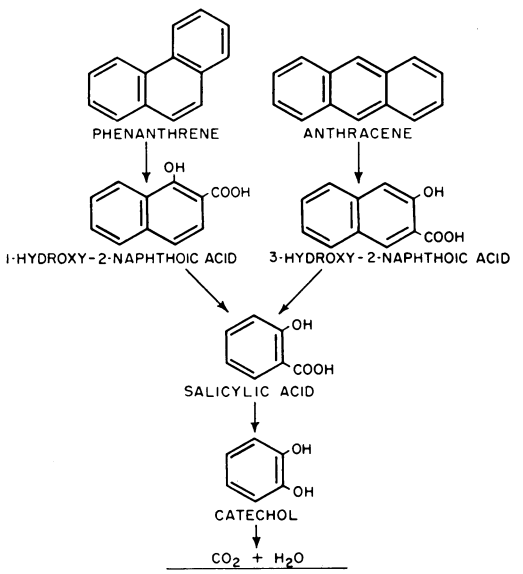

Figure 2. Pathways of polycyclic hydrocarbon oxidation.

by a bacterium resembling *Pseudomonas aeruginosa*. This product results from attack at an end ring as opposed to splitting of the reactive 9:10-bond as postulated by Tausson (Planta, **5**, 239, 1938). Studies on dissimilation of anthracene were carried out to determine the mode of attack on a linearly condensed polycyclic system as opposed to the angularly condensed phenanthrene molecule.

The anthracene-decomposing organism, a gram negative motile rod, was isolated from soil by enrichment culture with naphthalene as a source of carbon. The organism closely resembles *Pseudomonas aeruginosa* and differs from the phenanthrene strain only in its inability to produce acid from glucose. Growth of the culture in serial transfers in liquid mineral-salts medium containing anthracene as a source of carbon, indicated the ability of the organism to utilize anthracene. After an incubation period of 2 to 3 weeks at room temperature on a shaker, aliquots of anthracene fermentations gave a faint green color with ferric chloride, indicating a phenolic intermediate. In several experiments, pairs of 4-L flasks containing 800 ml of mineral-salts medium and 0.5 per cent anthracene were inoculated with 100 ml of a broth culture, followed by incubation for 10 days on a rotary shaker (80–90 rpm). The cultures were removed from the shaker and allowed to stand at room temperature until aliquots gave a green color with ferric chloride, usually after an additional 2–3 weeks. The cultures were then neutralized with 5.0 per cent $NaHCO_3$ and concentrated under reduced pressure to one-tenth volume. After acidification with 1:1 HCl, the residues were continuously extracted with ether for 48 hr. The ether solutions were extracted with 5.0 per cent aqueous $NaHCO_3$. The aqueous layer was separated, acidified, and centrifuged. The solid material obtained was recrystallized twice from hot water, yielding small amounts of yellow crystals. This material was identified as 3-hydroxy-2-naphthoic acid by comparison of its infrared and ultraviolet spectra with those of a known sample of the acid.

Manometric experiments with anthracene-grown cells demonstrated that the organism possessed the enzymes necessary to oxidize salicyclic acid and catechol (figure 1). The isolation of 3-hydroxy-2-naphthoic acid from anthracene breakdown, and of 1-hydroxy-2-naphthoic acid from phenanthrene breakdown indicate end ring attack as a mode of oxidation of polycyclic systems by members of the genus *Pseudomonas*. The pathways diagrammed in figure 2 are tentatively offered for phenanthrene and anthracene dissimilation.